

Insights of the Nitrosylation Pathway of [Fe-S] Proteins using Peptide-based Biomimetic Clusters

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Introduction

The study of the reaction of nitric oxide (NO) with Iron-Sulphur ([Fe-S]) proteins attract a lot of interest, because of the irreversible rearrangements of these metal-clusters, which is one of the major contributors to the toxicity of NO.^{1,2} Our study focused on the study of the reaction of NO with biomimetic [4Fe-4S] clusters coupled with IR spectroscopy (see Figure 1).

The biomimetic clusters were prepared using small peptides as ligands, with general sequence Ac-KC(A)₂₋₄CK-NH₂, where the alanine was used as spacers giving more conformational freedom for the iron(II) peptide coordination by cysteine residues, and the lysine to balance the electrical charge of the system. The [4Fe-4S] clusters were self-assembled in DMSO solution in the presence of triethylamine. The obtained clusters and their products of reaction with NO were characterized by FTIR, EPR and UV-Vis spectroscopy. Finally, the results of the nitrosylation reaction were also compared to the products of the [4Fe-4S] High Potential Iron Protein [HiPIP].

Results & Discussion

The obtained clusters were characterized by UV-Vis (absorption in 330 and 420 nm), electrochemistry ($E_{1/2red} = -0.96V$ and $E_{1/2ox} = 0.04V$ vs FeCp/FeCp⁺) and EPR ($g_z = 2.00$ and $g_{x,y} = 1.96$), those results suggests that the formed [4Fe-4S] clusters have electronic structure similar to those observed for the natural HiPIP, as reported previously.⁴

The reaction with NO was done for biomimetic [4Fe-4S] clusters, and the product reaction were monitored by FTIR by using an attenuated total reflectance (ATR) or a transmission dispersive, by using NO(g) flow cell or NO donor molecules. The FTIR results showed the presence of different species as intermediates for the reaction, leading to the formation of the Roussin's Black Salt (RBS), represented by the band at 1745 cm⁻¹ in IR spectrum (see Figure 1). The UV-Vis showed the formation of RBS in the end as well, absorption bands at 360 and 430 nm. The use of EPR gave insights about the possible intermediates of the reaction, principally showing the presence of dinitrosyl iron complexes in solution, with characteristic absorption band at $g = 2.03$. During the reaction others iron nitrosyl species were observed in the FTIR spectra. The use

of the two chosen peptides changed the pathway of the reaction with NO, where the cluster assembled with the peptide-containing six residues lead to the direct formation of RBS, and the other one with eight residues, presented more intermediates species and a slower reaction rate.³

The nitrosylation reaction was also carried out with HiPIP, by exposing the protein solution to a saturated NO(g) atmosphere. It showed that the formation of RBS is the major product of reaction. The presence of protein-bound Roussin's Red Ester (RRE) was also observed during the period.¹

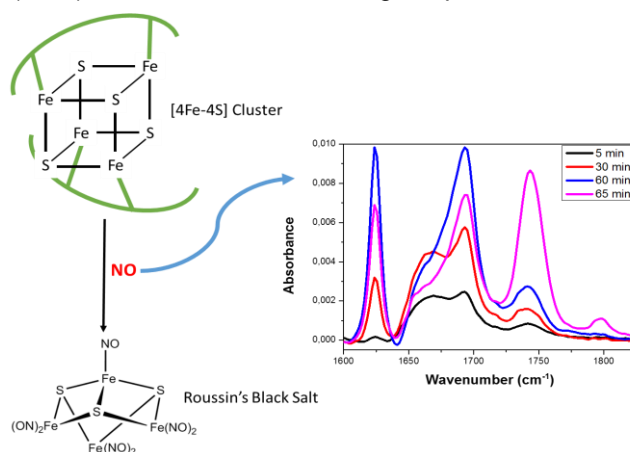


Figure 1. Scheme of the reaction of the biomimetic [4Fe-4S] clusters with NO leading to the RBS molecule at the end of the reaction. The graph show the time evolution of the Fe-NO bands in the FTIR spectra, after 65 min the RBS absorption at 1745 cm⁻¹ is the most prominent.

Conclusions

The obtained [4Fe-4S] clusters are biomimetic of HiPIPs. The use of biomimetic clusters coupled with IR spectroscopy, gave new insights about the nitrosylation reaction of [Fe-S] proteins, and that the major product of the reaction is the RBS for both types of compounds and for the protein HiPIP.

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¹ Grabarczyk, D. B.; Ash, P. A.; Vincent, K. A. *J. Am. Chem. Soc.* **2014**, *136*, 11236.

² Crack, J. C. *et al. Curr. Opin. Chem. Biol.* **2012**, *16*, 35.

³ Harrop T.C. *et al. J. Am. Chem. Soc.* **2008**, *130*, 15602.

⁴ Dey, A. *et al. Science.* **2007**, *318*, 1464.